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Surfing snails: Population genetics of the land snail *Ventridens ligera* (Stylommatophora: Zonitidae) in the Potomac Gorge*

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Abstract: The population structure of the land snail *Ventridens ligera* (Say, 1821) was investigated in the Potomac River Basin (Virginia, Maryland, U.S.A.). Animals were collected from two islands and the adjacent riverbanks along an 8.8-km stretch of the river. Four landlocked populations in Illinois and Maryland were also sampled to provide a comparison to the river populations. A total of 246 individuals were genotyped with five newly designed species-specific microsatellite primers. Low pairwise F_{ST} values (<0.0477) among the Potomac River sites suggest high levels of gene flow between the populations. In contrast, the landlocked populations had high F_{ST} values (0.0738 to 0.6004) which suggest genetic structuring, most likely due to physical isolation, because F_{ST} values >0.2 indicate population structuring. Low-level isolation by distance was found among the Potomac River populations, and the low FST suggests that the river is facilitating gene flow rather than acting as a barrier.

Key words: genetic diversity, microsatellite, isolation by distance

Distribution of genetic variation in natural populations often varies across a species range. Separation due to distance or physical barriers (*i.e.,* bodies of water, fragmented habitat) can further subdivide a species into local subpopulations (Selander and Kaufman 1975, Selander and Ochman 1983, McCauley 1995, Pfenninger *et al.* 1996, Arnaud *et al.* 1999a, 1999b). Isolation can often lead to lower genetic diversity and a higher risk of extinction (Crozier 1997, Saccheri *et al.* 1998, Allendorf and Luikart 2007).

Due to a limited ability to disperse, land snails tend to live in discrete populations with neighboring populations genetically more similar than distant populations (Pfenninger *et al.* 1996, Arnaud *et al.* 2001, Schweiger *et al.* 2004). Arnaud *et al.* (2001) found a positive correlation between geographic distance and genetic distance in the land snail *Helix aspersa* (Müller, 1774) sampled along a 900-m ditch in the polders of the Bay of Mont-Saint-Michel in France (F_{ST} = 0.055 to 0.02). Land snails isolated by water (*i.e.,* on islands) face a greater barrier to gene flow than distance. Gene flow may occur only during flooding, when debris carrying snails washes ashore, or if the snails are inadvertantly transported by other organisms. Migration of a river channel, connecting or separating pieces of land and the populations on them, may also facilitate or disrupt gene flow. Therefore land snail populations separated by barriers, such as rivers, would be expected to show the same or greater genetic isolation than populations in continuous environments.

Few studies have been done on land snails to assess the effect of physical barriers on gene flow. Arnaud *et al.* (2003) reported that the population sub-structuring found in *Helix aspersa* in the polders of the Bay of Mont-Saint-Michel was due to isolation by distance and not by habitat fragmentation as might be expected. My study focused on *Ventridens ligera* (Say, 1821) populations living along the riverbanks and on two islands in the Potomac River Basin. *Ventridens ligera* is a small (11-15.6 mm) snail in the subfamily Gastrodontinae (Burch 1962). Individuals are usually found in the leaf litter of wooded areas. The species range extends from New York to Florida and west to Michigan and Oklahoma. Species-specific microsatellite markers were developed to assess the spatial partitioning in *V. ligera* populations located along the Potomac River and to investigate whether the river acts as an effective barrier to gene flow.

MATERIALS AND METHODS

Sample collection

Ventridens ligera were collected at 14 locations along a 8.8-km stretch of the Potomac River north of Washington D.C., including Bear Island and Plummers Island, and both riverbanks adjacent to the islands located in the Chesapeake and Ohio Canal National Historical Park (Maryland) and George Washington Memorial Parkway/Great Falls Park

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(Virginia) (Fig. 1A-B). Samples were collected during the summer months in 2005 and 2007. Additional samples were collected on the campus of Southern Illinois University in Carbondale, Illinois and a site outside of Carbondale, Illinois (Fig. 1C), the campus of Towson University in Towson, Maryland and a site in southwestern Baltimore County, Maryland (Fig. 1A) in July 2008. Ten to twelve individuals were collected at each site. Samples were frozen at -80 °C upon return to the laboratory at Towson University. Alcohol preserved specimens of congeneric *Ventridens* were provided by the Field Museum of Natural History (Chicago, Illinois), the Carnegie Museum of Natural History (Pittsburgh, Pennsylvania), the Florida Museum of Natural History (Gainesville, Florida), and the Delaware Museum of Natural History (Greenville, Delaware).

Microsatellite marker development

Ten previously published land snail microsatellite markers (Guiller *et al.* 2000, Wirth 2000, Dépraz *et al.* 2008) were tested on *Ventridens ligera*, but all failed to amplify or give a clean product suitable for genetic analysis. Therefore, *Ventridens* specific primers were developed. Microsatellite markers were developed following the protocol described by Hamilton *et al.* (1999). Briefly, genomic DNA from five different *V. ligera* individuals was pooled and fragmented with restriction enzymes *Hae*III, *Rsa*I, and *Nhe*I. Fragments between 200-1000 bp in length were isolated by gel electrophoresis and ligated to special linker fragments that facilitate isolation and cloning. Probes containing microsatellite repeats were then hybridized to the linker-DNA assembly in order to isolate fragments that contained repeats. The resulting fragments were ligated into pBluescriptII KS plasmids (Stratagene, La Jolla, California) and the recombinants transformed into competent DH5α *Escherichia coli* cells. Plasmid DNA was extracted from clones positive for inserted DNA using a Qiagen MiniPrep Spin Plasmid Kit (Qiagen, Valencia, California). Insert DNA was sequenced on an ABI 3130 XL Genetic Analyzer with plasmid primers T7 and M13 Reverse at the Center of Marine Biotechnology (Baltimore,

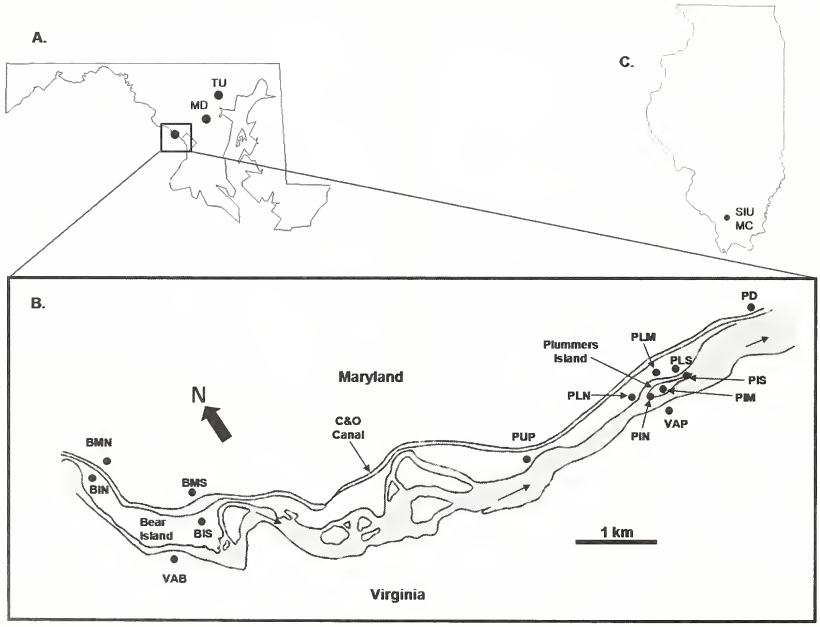

Figure 1. Map of collection sites. A, Map of the state of Maryland showing the location of collection sites TU, MD, and the Potomac River sites; B, map of the collection sites located in the Potomac River Basin below Great Falls; C, map of the state of Illinois showing the location of Carbondale and collection sites SIU and MC.

Maryland). Primers were designed for each microsatellite locus using Primer3 (Rozen and Skaletsky 2000). Plasmid DNA was purified from 305 bacterial colonies and sequences obtained for 125 plasmids containing appropriately sized inserts. Twenty of the sequences were used for primer development and five were ultimately found informative.

All five microsatellite loci were highly polymorphic (Table 1) with the number of alleles ranging from 17 at locus VL301 to 27 at locus VL10. Observed and expected heterozygosities for each locus ranged from 0.420 to 0.699 and 0.697 to 0.784, respectively. Primers were also tested on fourteen additional species of *Ventridens* and found to amplify comparably sized products in nine of the fourteen (Table 2).

Table 1. Characterization of five microsatellite loci in the land snail species *Ventridens ligera*. For each locus, we provide the repeat motif, the primer sequences, the observed allele range, the species-specific annealing temperatures for PCR, the number of alleles (Na) identified in our study population, the observed heterozygosity (H_o), and the expected heterozygosity (H_e).

Locus	Repeat motif	Product range (bp)	T_A (°C)	Primer sequences	Sample size	Na	H_o	H_e
VL10	$(GTT)_{20}(GAT)_2(GTT)_4(GAT)_2$	263-377	60	F: 5'-GTTTGGGTGGCAGATCACTT-3' R: 5'-TCCAGCTGATTGTTCATTCG-3'	246	27	0.574	0.697
VL1A	$(TCA)_{29}$	223-288	60	F: 5'-GGACCAACTCCTGTTGCATT-3' R: 5'-CTCTTCTTCGATGGCGAATC-3'	246	23	0.699	0.752
VL542	$(CAT)_{21}$	134-215	70-60*	F: 5'-GCCGTGTAAAAGACGACGA-3' R: 5'-CACCTACGGTAATCGGCACT-3'	246	22	0.420	0.762
VL301	$(GT)_4(GTT)_{22}TGATGGTT(GT)_3(GTT)_5$	212-262	58	F: 5'-GCTTCAGTTTTCAGGGCATC-3' R: 5'-GGGTCCTGGACTCATAGCAA-3'	246	17	0.640	0.724
VL306	$(CAA)_{35}TAACAA(TAA)_2(CAA)_{27}$	224-338	60	F: 5'-GATCGCGCCTTCAAATAACT-3' R: 5'-CGACCCGTCACCTAGGATCT-3'	246	25	0.597	0.784

* Touchdown PCR cycle, T_A decreased by 2 °C every 5 cycles until 60 °C

DNA preparation

Genomic DNA was purified from a small piece of foot tissue following the protocol described in Miller *et al.* (2000). A 2-4 mm length of foot was removed with a sterile razor blade and placed in 460 µl of extraction buffer (10 mM Tris pH 8.0, 50 mM EDTA, 1% SDS) along with 30 µl Proteinase K (10 mg/ml – final concentration 0.6 µg/µl) and 10 µl 0.1M DTT. Samples were incubated at 55 °C, 225 rpm for 2-3 h. Once the tissue had been completely digested, 5M NaCl was added to a final concentration of 1.5M and the sample incubated at 55 °C, 225 rpm for 30 min. Samples were centrifuged for 10 min at 0.87 RCF to pellet any remaining undigested material or debris and the supernatant transferred to a sterile microcentrifuge tube. An equal volume of chloroform was added and the mixture incubated at room temperature for 30 min at 100 rpm on an orbital shaker. The mixture was then centrifuged at 0.87 RCF for 10 min. The aqueous phase of the suspension was transferred to a sterile microcentrifuge tube and an equal volume of isopropanol was added. The tube was inverted several times to mix and centrifuged at 0.94 RCF for 15 min to pellet the DNA. Following centrifugation, the DNA pellet was washed with 70% ethanol, air-dried, and re-suspended in 75 µl Tris-EDTA buffer pH 8.0.

Microsatellite genotyping

Individual genotypes were obtained using five polymorphic microsatellite loci (Table 1). Polymerase chain reaction (PCR) was conducted in 15 µl volumes under the following conditions: 1X PCR buffer (Gene Choice, Continental Lab Products, San Diego, California), 200 µM dNTPs (Roche, Indianapolis, Indiana), 0.2 µM of each primer, 1.25 units of *Taq* polymerase (Gene Choice), and 30 ng of template DNA. The PCR thermocycling program consisted of 94 °C for 3 min, 35 cycles of 94 °C for 30 s, T_A (dependent on primer pair, Table 1) for 30 s, and 72 °C for 45 s followed by a final extension of 72 °C for 5 min. Primers for locus VL542 required a touchdown PCR with the T_A stepping down 2 °C from 70 °C to 62 °C with 5

Table 2. Amplification of microsatellite loci in other species of *Ventridens*. The symbol + indicates that a product was amplified and verified by gel electrophoresis and fragment analysis. Locus 542 failed to amplify in all species examined.

Species	VL10	VL1A	VL301	VL306	VL542
V. acerra (J. Lewis, 1870)	+	+	+		
V. arcellus (Hubricht, 1976)	+	+	+	+	
V. brittsi (Pilsbry, 1892)	+	+	+	+	
V. cerinoideus (Anthony, 1865)		+			
V. collisella (Pilsbry, 1896)	+	+			
V. demissus (A. Binney, 1843)	+	+	+	+	
V. intertextus (A. Binney, 1841)		+			
V. lawae (A. Binney, 1892)		+	+		
V. volusiae (Pilsbry, 1900)	+	+	+	+	

cycles at each step and 20 cycles at 60 °C; the rest of the program was the same. PCR products were verified by gel electrophoresis on a 1.5% agarose gel with ethidium bromide. The forward primer for each locus was labeled with a fluorescent dye (Sigma Proligo, St. Louis, Missouri) to facilitate fragment analysis on a CEQ8000 Genetic Analysis System (Beckman Coulter, Fullerton, California).

Statistical analysis

The number of alleles (N_a), expected heterozygosity (H_e), observed heterozygosity (H_o) and allelic richness (R_S) were calculated for each population and locus using GeneAlex6 (Peakall and Smouse 2006) following the methods of Hartl and Clark (1997).

An analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) was used to determine partitioning of genetic variation within the region, among populations, and among individuals within populations from microsatellite allele frequencies according to Weir and Cockerham (1984) using Arlequin ver. 3.01 (Excoffier *et al.* 2005). Population structure was also tested using STRUCTURE v2.1 (Pritchard *et al.* 2000) with a burn in period of 5,000 and 50,000 reiterations after burn in. STRUCTURE uses a Bayesian approach to assign individuals to clusters based on their genotypes while estimating population (cluster) allele frequencies (Pritchard *et al.* 2000).

Global and pairwise F_{ST} (Weir and Cockerham 1984) values were calculated using FSTAT v2.9.3 (Goudet 2001) to measure population differentiation. Significance was determined using 1,000 permutations with Bonferroni multiple corrections at the 1/1,000 nominal level in FSTAT. To test for population-level deviations from Hardy-Weinberg equilibrium, Wright's F_{IS} was calculated for each population using the estimator θ (Weir and Cockerham 1984) in GENETIX v4.05 (Belkhir *et al.* 2004). Significance of F_{IS} was tested with 1,000 permutations.

Isolation by distance was tested using a Mantel test of the association between genetic distance and the logarithm of geographic distance in the IBD program (Bohonak 2002). Genetic distance was calculated as $F_{ST} / (1 - F_{ST})$ and geographic distances (km) were based on GPS coordinates. The Mantel test was based on 10,000 permutations of spatial locations among the sample populations.

RESULTS

Population analysis

Levels of polymorphism in each population varied with the average number of alleles ranging from 2.2 in the TU population to 11.6 in the PLN population (Table 3). Allelic richness (R_S), which accounts for sample size biases, showed little difference among the Potomac River populations with values ranging from 5.4210 to 6.2102. In contrast, R_S for the four landlocked populations ranged from 1.8704 to 3.9520. Average observed (H_o) and expected (H_e) heterozygosities for the Potomac River populations ranged from 0.4666 to 0.7810 and 0.7692 to 0.8496, respectively. Values for the landlocked populations ranged from 0.2466 to 0.3820 (H_o) and 0.2600 to 0.6774 (H_e). Nine of the 18 populations had significantly positive multilocus F_{IS} values indicating an overall heterozygote deficiency.

AMOVA results estimated the global F_{ST} for all 18 populations to be 0.091 ($P < 0.0001$) (Table 4). Analysis of all populations found that 70% of the variation was among the individuals of the populations whereas only 9.11% was between the populations. A separate analysis of the 14 Potomac River populations found that 79.33% of the variation was among individuals in contrast to 1.64% among the populations. Analysis of the four landlocked groups alone showed that 43.88% of the variation was among the individuals with 34.86% among the populations.

Pairwise analysis of F_{ST} estimates between the Potomac River populations (Table 5), resulted in values ranging from -0.0003 to 0.0477. Pairwise analysis of the four landlocked populations with the Potomac River populations and each other resulted in F_{ST} estimates ranging from 0.0738 to 0.6004. F_{ST} values for several Potomac River populations were significant; however, no patterning was evident (*i.e.,* island sites vs. mainland sites) whereas nearly every value for the four landlocked sites was significant. Analysis by STRUCTURE clearly delineated the samples into three clusters: the Potomac River sites, the Illinois sites (SIU, MC), and the Maryland sites (TU, MD) (data not shown).

Based on the Mantel test, there was a significant association between the pairwise genetic and geographic

Table 3. Intrapopulation genetic diversity at five microsatellite loci for 18 *Ventridens ligera* sampling sites. For each population I provide the sample size, average number of alleles (N_a), the observed heterozygosity (H_o), the expected heterozygosity (H_e), F_{IS}, which indicates deviation from random mating, and allelic richness (R_s). * Indicates significant values.

Population	Sample size	N_a	H_o	H_e	F_{IS}	R_s
PIS	18	10.4	0.7056	0.8018	0.1490*	5.7526
PIM	19	9.6	0.5632	0.8004	0.3218	5.6158
PIN	19	9.8	0.6032	0.7842	0.2572	5.4210
PLS	19	10.0	0.6892	0.8232	0.1906*	5.7702
PLM	21	10.0	0.6704	0.8052	0.1914	5.6662
PLN	21	11.6	0.7810	0.8218	0.0740*	5.8826
PUP	11	9.0	0.6096	0.8232	0.2067	6.0056
PD	9	9.0	0.7684	0.8288	0.1367*	6.4610
VAP	9	6.8	0.6402	0.8092	0.2787*	5.6228
BMN	10	7.6	0.7200	0.8036	0.1568*	5.6386
BMS	8	7.6	0.6250	0.7672	0.2489*	5.8708
BIS	15	8.8	0.4666	0.8022	0.4463	5.5286
BIN	7	6.4	0.7428	0.7818	0.1261*	5.4942
VAB	18	10.6	0.5706	0.8496	0.3548	6.2102
SIU	10	2.8	0.3288	0.4678	0.3455	2.6040
MC	11	5.2	0.3510	0.6774	0.5194	3.9520
TU	10	2.2	0.2466	0.2600	0.1066*	1.8704
MD	11	3.8	0.3820	0.6768	0.4737	3.4042

distances between the sampled populations ($R^2 = 0.0599$, $P = 0.0281$). The plot of $F_{ST} / (1 - F_{ST})$ against log (geographic distance) (Fig. 2) shows a statistically significant positive relationship between the geographic distance and genetic similarity of the *Ventridens ligera* populations sampled ($P < 0.001$, 10,000 permutations).

DISCUSSION

We analyzed genetic variation in land snail, *Ventridens ligera*, populations along the Potomac River to determine if the river acts as an effective barrier against gene flow. Analysis of five *V. ligera* specific microsatellite loci showed no partitioning of genetic variation between individuals on Bear Island, Plummers Island, or the adjacent riverbanks but did show strong partitioning of individuals among the four landlocked sites (SIU, MC, TU, and MD). These results suggest that *V. ligera* in the Potomac River populations experience higher levels of gene flow than the landlocked populations.

The global F_{ST} estimate among the 18 populations was low ($F_{ST} = 0.091$; $P < 0.0001$) which suggests a lack of genetic partitioning and that gene flow is occurring between the sampled populations. However, analysis of the Potomac River populations and the landlocked sites separately revealed F_{ST} values of 0.016 (P not significant) and 0.350 ($P < 0.0001$), respectively. F_{ST} values >0.2 are considered to reflect population structuring (Beebee and Rowe 2008). The F_{ST} for the Potomac River populations only is much lower than the value when all populations or the landlocked populations only are evaluated, further supporting the suggestion that gene flow is occurring among these river populations. In contrast, the F_{ST} for the landlocked sites suggests that there is structuring between the four populations and there is no gene flow occurring. Analysis in STRUCTURE found no partitioning among the Potomac River populations; instead the results suggest that all 14 sites are part of one large homogenous population.

Pairwise F_{ST} values were very low (-0.0003 to 0.0483) among the Potomac River populations while values for the landlocked sites ranged from 0.0738 to 0.6004 with nearly every value significant. These results also indicated that the four landlocked populations were significantly different not only from the Potomac River populations but also from each other, while the Potomac Rivers populations are highly similar to each other. A Mantel test showed a significant positive correlation between genetic distance and geographic distance. Over short distances (<1 km) there is a linear pattern of isolation that is expected as gene flow homogenizes the populations. The pairwise comparisons that fall within this range are those on the same islands (*i.e.*, Plummers Island to Plummers Island, Bear Island to Bear Island). The more distant comparisons (>2 km; *i.e.*, Bear Island to Plummers Island) display a significant level of isolation by distance most likely due to a limited gene flow and genetic drift.

Table 4. Analysis of molecular variance (AMOVA) describing the partitioning of genetic variation for 18 *Ventridens ligera* sampling sites. All samples were analyzed then reanalyzed in two groups: Potomac River populations and the landlocked populations.

Source - All 18 populations	*d.f*	Estimated variance	Percentage
F_{ST}			
Among populations	17	0.154	9.11
Among individuals			
Within populations	228	0.352	20.77
Within individuals	246	1.189	70.12
Total	491	1.696	100
Global $F_{ST} = 0.091$, $P < 0.0001$			
Source - Potomac River only			
F_{ST}			
Among populations	13	0.027	1.64
Among individuals			
Within populations	190	0.317	19.03
Within individuals	204	1.324	79.33
Total	407	1.668	100
Global $F_{ST} = 0.016$, $P = 0.934 \pm 0.006$			
Source - 4 landlocked sites			
F_{ST}			
Among populations	3	0.586	34.86
Among individuals			
Within populations	38	0.358	21.26
Within individuals	42	0.738	43.88
Total	83	1.682	100
Global $F_{ST} = 0.3486$, $P < 0.0001$			

The Potomac River Basin floods frequently and may act as a catalyst for unidirectional gene flow. It is highly possible that *Ventridens ligera* from upstream are "rafting" on debris downstream during both normal and flooding periods. Chiappero *et al.* (1997) reported this phenomenon in the sigmodontine rodent *Oligoryzomys flavescens*. The authors reported a downstream flow of genes over 250 km of the Paraná River in Argentina with the highest heterozygosity found at the site furthest upstream. They suggest that the animals may be passively transported downstream on rafts of floating plants. This method of gene flow for *V. ligera* is certainly possible, and a larger study to further elucidate gene flow is planned with sampling from populations near the source of the Potomac River, at the mouth of the river where it enters the Chesapeake Bay, and at sites along the length of the river. While I did identify upstream sites with higher

Table 5. Pairwise F_{ST} estimates of *Ventridens ligera* populations based on five microsatellite loci. Bold values indicate significant differences at $P < 0.05$.

	PIS	PIM	PIN	PLS	PLM	PLN	PUP	PD	VAP	BMN	BMS	BIS	BIN	VAB	SIU	MC	TU	MD
PIS	---	0.0288	0.0264	0.0134	0.0298	**0.0328**	00164	0.0483	0.0120	**0.0208**	0.0403	0.0270	**0.0477**	0.0232	**0.2795**	**0.1598**	**0.3400**	**0.1113**
PIM	---		-0.0044	0.0155	0.0161	0.0123	0.0024	0.0294	0.0209	0.0233	0.0243	**0.0327**	0.0188	0.0122	**0.2937**	**0.1667**	**0.2891**	**0.1269**
PIN			---	0.0057	0.0148	0.0071	-0.0019	0.0177	0.0312	0.0189	0.0134	0.0254	0.0228	0.0051	**0.2941**	**0.1624**	**0.2936**	**0.1220**
PLS				---	-0.0055	-0.0022	-0.0095	-0.0005	0.0070	0.0200	0.0226	0.0159	0.0164	0.0120	**0.2612**	**0.1534**	**0.3076**	**0.1186**
PLM					---	0.0083	0.0155	0.0376	0.0212	0.0352	0.0464	0.0162	0.0137	0.0228	**0.2787**	**0.1581**	**0.3201**	0.1317
PLN						---	-0.0016	0.0188	0.0147	0.0280	0.0373	0.0160	**0.0356**	0.0086	**0.2762**	**0.1644**	**0.2904**	**0.1015**
PUP							---	-0.0023	0.0042	0.0098	0.0209	0.0149	0.0148	0.0106	**0.2802**	**0.1662**	**0.3352**	**0.1090**
PD								---	0.0115	0.0282	0.0169	0.0268	0.0298	-0.0003	0.2734	0.1316	0.3262	**0.0979**
VAP									---	0.0126	0.0261	0.0127	-0.0104	-0.0029	**0.2519**	**0.1303**	**0.3881**	**0.0908**
BMN										---	0.0294	0.0291	0.0288	0.0151	**0.2876**	**0.1759**	**0.3833**	**0.1249**
BMS											---	0.0124	0.0334	0.0058	**0.3055**	**0.1631**	**0.3840**	**0.1384**
BIS												---	0.0097	0.0082	**0.2632**	**0.1775**	**0.3427**	**0.1167**
BIN													---	0.0201	**0.2654**	**0.1669**	**0.3955**	**0.1251**
VAB														---	**0.2554**	**0.1318**	**0.2885**	**0.0738**
SIU															---	0.2739	**0.6004**	**0.3394**
TU																---	**0.4083**	**0.1930**
MD																	---	**0.3549**

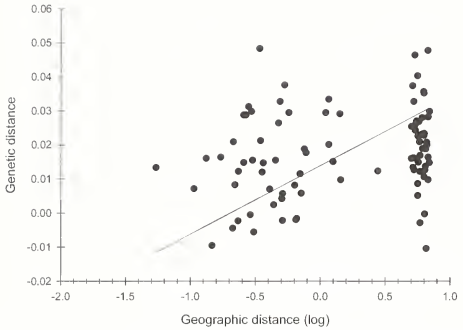

Figure 2. Genetic distance vs. log(geographic distance) in *Ventridens ligera* populations along the Potomac River. Genetic distance is shown as $F_{ST} / (1 - F_{ST})$. Circles refer to all possible pairwise comparisons among the Potomac River populations.

heterozygosity than downstream sites, my findings were not consistent. I suggest this is potentially due to the small distance analyzed, and that the study spanning the entire Potomac River will show higher heterozygosities upstream near the source.

These are rather small land snails and the cost of locomotion across even several meters would be extremely high. We collected samples along an 8.8 km stretch of the Potomac River and it is highly doubtful that *V. ligera* are traveling this distance on land. Land snails typically migrate only a few meters per year (Pfenninger *et al.* 1996, Arnaud *et al.* 1999b). However, passive displacement (*i.e.,* other animals, water) could potentially move individuals a much larger distance (Dörge *et al.* 1999). Previous land snail studies have shown that land snails live in neighborhoods where populations are genetically distinct from one another (Selander and Kaufman 1975, Pfenninger *et al.* 1996, Arter 1990, Johnson and Black 1995, Arnaud *et al.* 2001). Pfenninger *et al.* (1996) used RAPD analysis to determine that the land snail *Trochoidea geyeri* (Soós, 1926) lived in genetically distinct neighborhoods of 13-21 m^2. Arnaud *et al.* (2001) reported significant genetic structuring between neighborhoods of *Helix aspersa* only 40 m apart using microsatellites. Both of these studies focused on populations located in habitats without moving water.

Here I report the first evidence of gene flow among land snails in the Potomac River Basin. My findings suggest that the river is not an effective barrier to gene flow in the land snail *Ventridens ligera*. Evidence of isolation by distance between the populations on two separate islands is present; however, because the overall F_{ST} values are close to zero, I suggest that the river is actually facilitating gene flow.

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